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Induction and subcellular localization of protein kinase C isozymes following renal ischemia

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Induction and subcellular localization of protein kinase C isozymes following renal ischemia.

Background. We have previously reported that the expression of the receptor for activated C kinase (RACK1) is induced post-ischemia/preperfusion injury to the kidney, and activation of protein kinase C (PKC) protects renal cells from hypoxic injury. This study was done to determine whether the induced expression of RACK1 is accompanied by changes in the level of expression and subcellular distribution of PKC isozymes.

Methods. Ischemia/reperfusion injury resulting in acute renal failure was induced by 60 minutes of bilateral renal artery clamping in rats. The expression levels and translocation of various PKC isozymes between soluble and particulate fractions in whole kidney homogenates were demonstrated by immunoblot analysis. The expression pattern of the various PKC isozymes in the kidney postinjury was performed by immunohistochemistry.

Results. PKC α, βII, and ζ were induced and translocated from the soluble fraction to the particulate fraction post-injury. Immunolocalization showed PKC α, βII, and ζ expression to be induced in the proximal tubule epithelial cell (PTEC) at 0 to 30 minutes post-ischemia/reperfusion injury (IRI). At one-day postinjury, the α isozyme was translocated to the plasma membrane of the undamaged PTEC, while it was translocated to the nucleus in damaged PTEC. PKC βII expression was along the basal and lateral side of the undamaged PTEC, while it was distributed in the cytoplasm of sloughed cells in the damaged PTEC. PKC ζ expression at one day was along the apical side of the damaged PTEC. At seven-days postinjury, the expressions of the α and ζ isozymes were localized to the plasma membrane of the regenerating PTEC and the expression of PKC βII isozyme to certain interstitial cells.

Conclusion. The induced expression, translocation, and the intracellular spatial distributions of the enzymes suggest that they may mediate multiple processes during IRI.

Reperfusion of the ischemic kidney triggers several molecular pathways, which eventually determine the fate of an injured cell [1]. A renal epithelial cell may die or survive. Those that survive dedifferentiate and enter the cell cycle to repopulate the denuded areas of the tubule [2]. The pathophysiological consequences as well as the dedifferentiation and proliferative responses during the tissue repair phase depend on molecular responses mediated by various signal transduction pathways [3]. The identities of such pathways are largely unknown.

Protein kinase C is a critical component of intracellular signal transduction pathways regulating a number of cellular events. These include homeostasis, migration, proliferation, apoptosis, remodeling of the actin cytoskeleton, and modulation of ion channels [4]. Protein kinase C (PKC) is a family of protein kinases that specifically phosphorylate Ser/Thr. The family includes at least 11 isozymes—α, βI, βII, γ, δ, ϵ, η, θ, ζ, ι, and μ—that are divided into three subgroups based on their structure and mode of activation. The classic or conventional PKCs (cPKC) α, βI, βII, and γ are activated by Ca^{2+}, phosphatidyl serine (PS), and diacylglycerol (DAG) or phorbol esters. The new PKCs (nPKC) δ, ϵ, η, and θ are activated by PS and DAG but not by Ca^{2+}. The atypical PKCs (aPKC) ζ and ι are dependent on PS, but are not activated by DAG, phorbol esters, or Ca^{2+} [4, 5]. Each of the isozymes has similar kinase regions. However, major differences exist in their developmental expression, tissue distribution, regulatory properties, and susceptibility to activators and inhibitors.

Protein kinase C is present in the mammalian kidney cortex [6], renal brush border membrane [7], and the basolateral membrane of the renal proximal tubules [8]. The distribution and relative abundance of the α, β, δ, and ζ isoforms in infant and adult kidneys have been described. Among the classic isoforms, PKC α isoform is the only one expressed in relative abundance in both infant and adult rat kidneys. In situ hybridization and immunohistochemistry show it to be distributed mostly to the S_3 segments of the proximal tubule located in the outer stripe of the outer medulla [9]. Levels of expression for PKC β are low. It is distributed in the medulla but not in the cortex. Among the members of the nPKCs, the δ isoform is expressed more in the medulla of the

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infant kidney as compared with the cortex. Among the aPKCs, PKC ζ is expressed in both infant and adult kidneys. Thus, four isoforms of PKC are expressed in the kidney. Since they have different responses to DAG, Ca^{2+}, and phospholipids, it seems likely that they are activated by different first messengers [10].

The identity and spatial distribution of various PKC isozymes that are induced and activated in acute renal injury have been partially analyzed. In an earlier report, La Porta and Comolli reported that following 30 minutes of ischemic renal injury, enhanced expressions of PKC α, δ, and ϵ and increased activity of PKC α occurred at 60 minutes post-reperfusion [11]. In a model of folic acid-induced renal injury, down-modulation of PKC α expression was described. Renal expression of the δ and ϵ isoforms was not changed following folic acid injury. No change in PKC α levels was found to be associated with focal necrosis of the S_3 segment and acute renal failure induced by the toxin S-(1,2-dichorovinyl)-L-cysteine(35) [10]. These studies suggest that individual PKC isozymes play distinct roles in the pathophysiology of the kidney following acute injury. However, the role of PKC isozymes has not been studied beyond two-hour post-IRI, and their translocation and spatial distribution have not been analyzed.

We have previously reported that the expression of the receptor for activated C kinase (RACK1) is induced post-ischemia/reperfusion injury to the kidney [12]. RACK1 is an anchoring protein for the activated form of PKC and serves to localize the enzyme in proximity to its substrates. The PKC-RACK1 complex has more potent kinase activity than activated kinases alone, suggesting that the complex formation may be the active form of the kinase in vivo [13].

We also have shown that activation of PKC isozymes in the kidney proximal tubular cell line LLCPK1 prior to oxidant injury protects cells from necrotic cell death [14]. Taken together, these two studies suggested the participation of PKC in various signal transduction pathways mediating the injury and repair processes of the kidney post-IRI.

This study surveyed the pattern of expression of individual PKC isozymes, their distribution between particulate and soluble fractions, and their subcellular distributions at various times postischemic injury in the rat kidney. PKC α, βII, and ζ are induced post-injury and are translocated to subcellular locations.

METHODS

Rat model of acute renal failure

Male Sprague Dawley rats (Harlan, Indianapolis, IN, USA) weighing approximately 225 to 249 g were used in the experiments. Ischemia/reperfusion (I/R) injury resulting in acute renal failure was induced by 60 minutes of bilateral renal artery clamping as described previously. The body temperature of the animals was maintained at 34°C to 38°C post-surgery during the recovery period. Blood was collected through a tail vein every 24 hours post-injury for seven days, and measurement of creatinine and blood urea nitrogen was performed as in prior studies [15, 16]. The animals were sacrificed at different time points postinjury, and both kidneys were processed for histologic and protein analysis. All animal studies were conducted in accord with the National Institutes of Health guide for the care and use of laboratory animals.

Western blot analysis

Kidneys from sham-operated and acute renal failure-induced rats were collected and homogenized in 3 to 5 mL per gram of tissue in RIPA buffer (142.5 mmol/L KCl, 5 mmol/L $MgCl_2$, 10 mmol/L HEPES, 1% NP40 and 1 × complete proteinase inhibitor; #1697498; Boehringer Mannheim, Germany). The homogenates were sonicated for three 30-second bursts on ice and centrifuged at 500 × *g* to remove cell debris. The homogenates were incubated for 20 minutes at 4°C on a rotating shaker. The amount of protein in each fraction was determined by the Bio-Rad protein assay system. Fifty micrograms were suspended in 10 μL of loading buffer and boiled for three minutes. The protein was run on a 4 to 20% gradient polyacrylamide gel and electrophoretically transferred to enhanced chemiluminescent (ECL) membranes. The membranes were blocked in TBST buffer (50 mmol/L Tris HCl, pH 7.5, 150 mmol/L NaCl, 0.05% Tween 20) containing 5% milk powder for one hour at room temperature. The blocking solution was then replaced with the same buffer containing individual PKC isozyme-specific antibody and incubated overnight at 4°C. The membrane was then washed three times in TBST and incubated with horseradish peroxidase-linked mouse or rabbit IgG for one hour at room temperature. Membranes were washed with TBST, and chemiluminescent detection was performed using the ECL detection kit (Amersham-Pharmacia Biotech, Inc., Piscataway, NJ, USA) according to the manufacturer's instructions.

Quantitation of the expression of the various PKC in soluble versus particulate fraction following ischemia

Kidney samples from various time points were minced and homogenized as described previously in this article. Supernatants were centrifuged at 100,000 × *g* for 30 minutes, and the resulting supernatants (soluble fraction) and pellets (particulate fraction) were collected. Protein concentration in each fraction was determined as described previously in this article. Fifty micrograms from each fraction derived from kidneys originating in sham-operated rats and rats rendered ischemic were run on gradient polyacrylamide gels, and Western blot analysis for various PKC isozymes was performed as described

previously in this article. The intensity of each band was quantitated using Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA) and expressed as a percentage of the intensity of the band derived from sham-operated animal (control) for each individual blot.

Immunohistochemistry

Localization of PKC isozymes was performed on 5 μm Bouin's fixed, paraffin-embedded sections. Following deparaffinization in xylene, tissue was microwaved in 0.01 mol/L bicarbonate for Bouin's fixed tissues or 0.01 mol/L citrate buffer for formalin-fixed tissues for antigen unmasking as previously described [17]. The tissues were then treated with 0.6% H_2O_2 in 80% methanol, and sequential incubations were carried out in solutions containing avidin and biotin (Zymed, San Francisco, CA, USA), respectively. The tissues were blocked for one hour in blocking buffer [0.01 mol/L phosphate-buffered saline (PBS), pH 7.4, containing 0.3% Triton X-100, 10% normal goat serum, 0.3% bovine serum albumin (BSA), and 100 mg/mL goat γ globulin). The primary PKC isozyme-specific antibodies were applied in the 1/10th concentration of the blocking buffer at 4°C overnight. Detection was performed using a streptavidin-biotin immunoperoxidase technique with aminoethylcarbozol (AEC) as a substrate (Histostain SP kit; Zymed). The specificity of staining was verified by performing control experiments in which tissues were incubated with the respective immunoglobulin subtypes.

Proliferating cell nuclear antigen staining

Proliferating cell nuclear antigen (PCNA) was used as a marker for cell proliferation; PCNA was detected using a PCNA staining kit (Zymed). The kit uses a biotinylated PCNA monoclonal antibody. Streptavidin-peroxidase was used as the signal generator. Color development was done using diaminobenzidine (DAB) as the chromogen to stain PCNA-containing nuclei dark brown.

Materials

The primary rabbit polyclonal antibodies for all of the PKC isozymes except for PKC α were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The primary mouse monoclonal antibody for PKC α was purchased from Upstate Biotechnology (Lake Placid, NY, USA).

RESULTS

Time course of relative abundance of PKC isozymes in kidney following ischemia

To determine the time course of expression of various PKC isozymes post-ischemic injury, kidneys were removed from rats that underwent 60 minutes of ischemia followed by various times of reperfusion. Fifty micrograms of protein from whole kidney were fractionated by polyacrylamide gel electrophoresis (PAGE). Western blot analysis was performed to determine the pattern of expression of all 11 PKC isozymes in the kidney immediately post-ischemic injury and during the reperfusion period. Protein extracts from brain (BR), and sham-operated rat kidneys (SH) were used as controls. Immunoreactivity was detected for PKC α, βII, δ, ϵ, and ζ. No expression of PKC βI, γ, η, θ, ι, or μ could be detected in the control or injured kidney fractions (data not shown). Representative immunoblots for the different isozymes that were expressed are shown in Figure 1. Sixty minutes of ischemia resulted in large increases in the expression of PKC α, βII, and ζ. The expression of each isozyme relative to levels in kidneys of sham-operated rats (SH) was induced at 0 minutes post-injury without reperfusion and was further up-regulated during the first one hour of reperfusion. The level of expression was decreased following one hour of reperfusion and was back to control levels (SH) by six hours post-reperfusion. The ex-

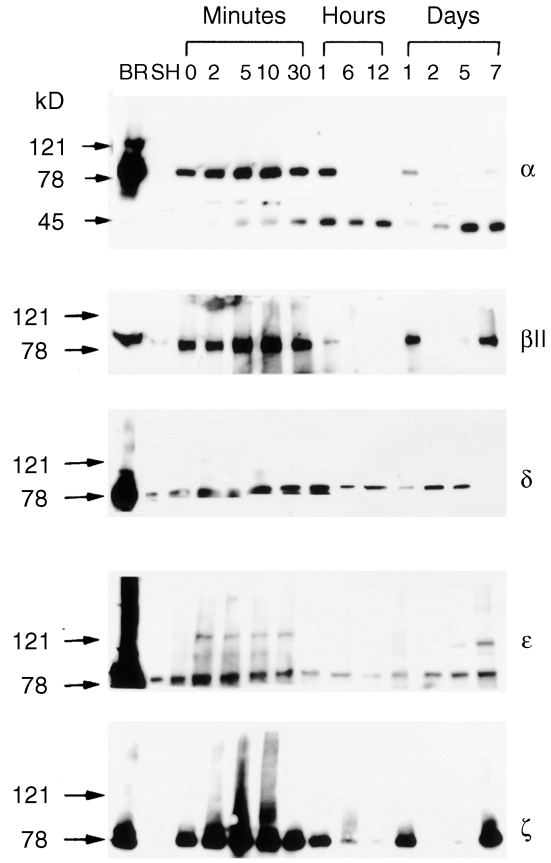


Fig. 1. Western blot analysis of protein derived from rat brain (BR), sham-operated (SH) rat kidneys and from rats that underwent 60 minutes of renal ischemia and various time periods of reperfusion. Fifty micrograms of protein extracted from the tissues were resolved on 4 to 20% polyacrylamide gels, transferred to enhanced chemiluminescence (ECL) membranes, and immunoblotted with various protein kinase C (PKC)-specific antibodies.

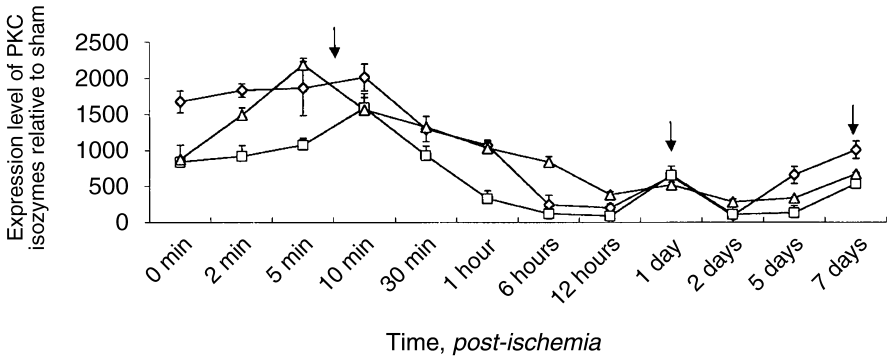


Fig. 2. Quantitation of the expression level of protein kinase C (PKC) α, βII, and ζ post-ischemic injury. Fifty micrograms of total protein lysates derived from three ischemic kidneys at various time points were immunoblotted to PKC α, βII, and ζ specific antibodies. The intensity of the bands was quantitated as described in the **Methods** section. Data for each time point are derived from three animals that underwent 60 minutes of ischemic injury followed by different reperfusion times. Abbreviations are: (◇), PKC-α; (□), PKC-βII; (△), PKC-ζ. Data are means ± SE.

pressions of PKC α, βII, and ζ increased relative to six hours post-reperfusion at one day and returned to control levels at two to four days. The expression was enhanced a third time (relative to 2 to 4 days) during five to seven days post-injury.

To quantitate the intensity of the bands, separate immunoblots were prepared from three rat kidneys for each time point. Quantitative data for the expression levels of the three isozymes PKC α, βII, and ζ are shown in Figure 2. The three peaks of activity (5 to 10 minutes, 1 day, and 7 days post-reperfusion) are illustrated with arrows.

Protein kinase C α activation has been shown to be regulated by its limited proteolysis catalyzed by calpain [18]. The decreased expression of PKC α post-30 minutes of reperfusion was found to be accompanied by an increase in the levels of its 45 kD proteolyzed fragment (Fig. 1).

No significant change in the level of expressions of PKC δ or ϵ compared with levels in the sham operated kidney was observed at any time post-injury.

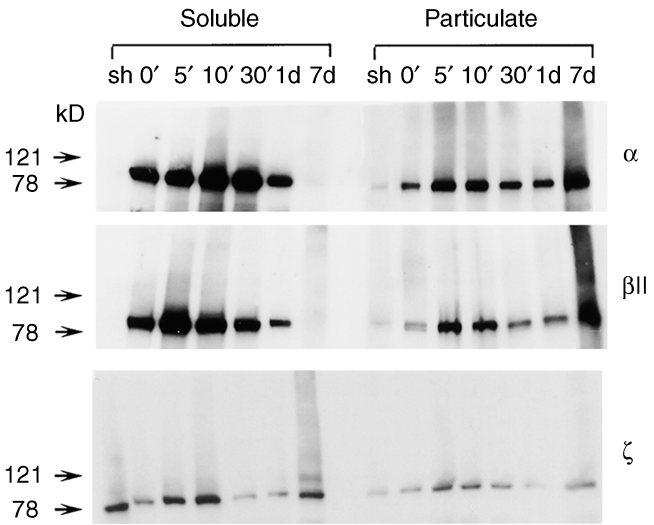


Fig. 3. Distribution of PKC isozymes in soluble and particulate fractions in kidneys derived from rats that underwent 60 minutes of renal ischemia and various time periods of reperfusion. Kidney homogenates from each time point were centrifuged at 100,000 × *g* to separate the soluble and particulate fractions. Fifty microgram protein aliquots from each fraction were resolved and immunoblotted as in Figure 1.

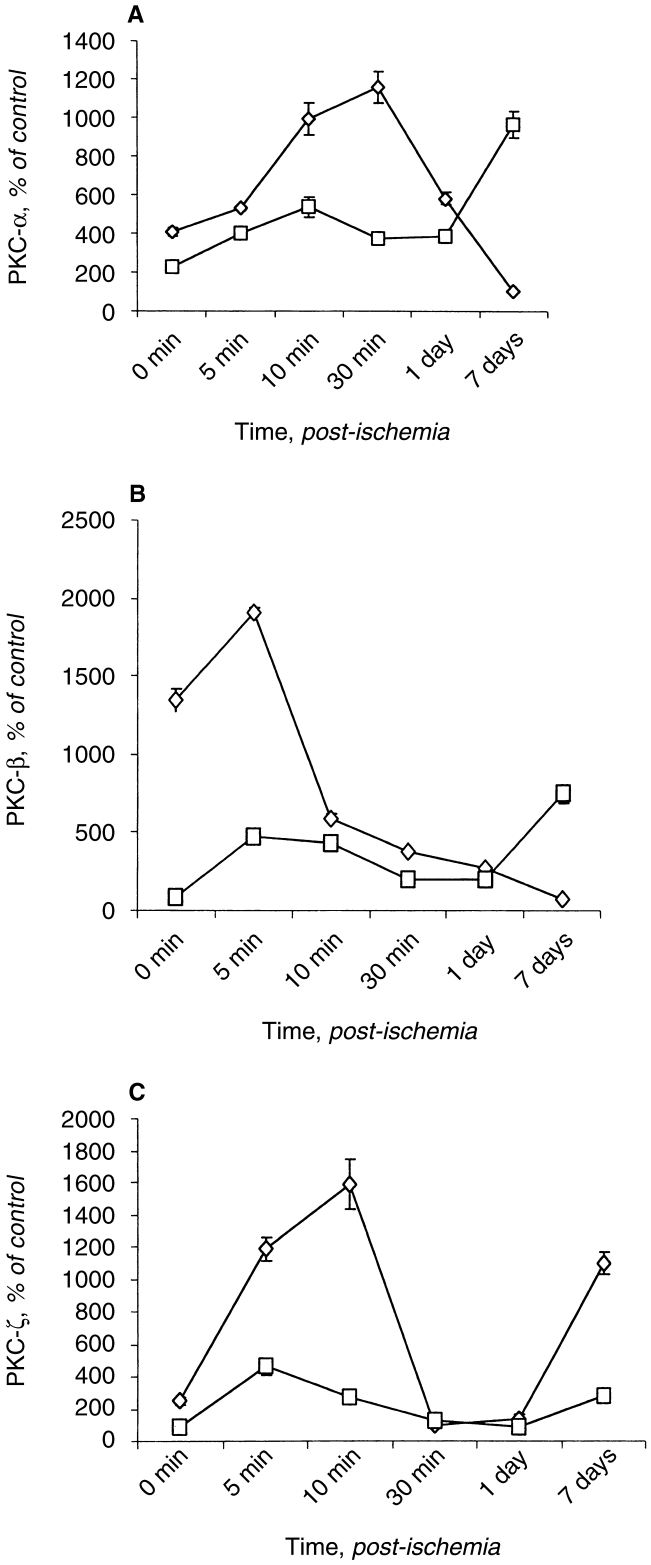
Translocation of specific PKC isozymes in the kidney post-ischemia/reperfusion injury

To evaluate whether the induced expression of PKC isozymes postinjury was accompanied by their translocation to membrane fractions, Western blot analysis was performed. Representative immunoblots for PKC α, βII, and ζ are shown in Figure 3. PKC α, βII, and ζ were translocated from cytosolic to particulate fraction beginning immediately after injury and were continued during the reperfusion period. Quantitative data of the distribution of the three isozymes between soluble and particulate fractions are shown in Figure 4. The maximal translocation to the particulate fraction for PKC α occurred between 10 and 30 minutes, for βII at 5 minutes, and ζ at 10 minutes post-reperfusion. At seven days post-injury, almost all of the PKC α, βII and ζ were found to be present in the particulate fraction. In contrast to the spatial translocation observed for PKC α, βII, and ζ, no significant change in translocation of PKC δ or ϵ was observed compared with sham-operated controls (data not shown).

Subcellular localization of PKC isozymes following renal ischemia

To characterize the cellular distribution of PKC isozymes α, βII, and ζ, immunocytochemical analyses were performed on kidney sections derived from various time periods post-injury. Kidney sections from sham-operated rats were used as controls. The localization of PKC α, βII, and ζ isozymes in kidneys from sham-operated rats (Fig. 5 A, D, and G) and in kidneys from rats rendered ischemic at 10-minutes post-reperfusion (Fig. 5 B, C, E, F, H, and I) are shown.

In kidneys from sham-operated rats, the PKC α isozyme was located in the brush border of the proximal tubules (Fig. 5A, arrow). In kidneys from rats rendered



ischemic, its expression was enhanced at the same location (Fig. 5B, arrow). A higher magnification of a small region from Figure 5B is shown in Figure 5C (arrow), demonstrating the localization of the PKC α isozyme in the brush border of the proximal tubule epithelial cell (PTEC). The absence of expression of PKC α in distal tubule is shown (Fig. 5C, arrowhead). The expression of PKC βII was localized predominantly in distal tubules in kidneys of sham-operated rats (Fig. 5D, arrowhead). The expression of PKC βII was absent in PTECs (Fig. 5D, arrow). However, its expression was highly induced in proximal tubules (Fig. 5E, arrow) and in distal tubules (Fig. 5E, arrowhead) post-injury. Higher magnification of a small region from Figure 5E demonstrates that PKC βII immunoreactivity was located predominantly to the basal and lateral side of the proximal tubular (PT) epithelial cells post-ischemia (Fig. 5F, arrow).

Protein kinase C ζ expression also was found in the distal tubules in the sham-operated rat kidneys (Fig. 5G, arrowhead). No expression of PKC ζ was observed in proximal tubules following sham injury (Fig. 5G, arrow). However, its expression was induced in the proximal tubular cells post-injury and was distributed along the apical side of the proximal tubule cells (Fig. 5H, arrow). The apical localization of PKC ζ isozyme is demonstrated by higher magnification (Fig. 5I, arrow) of a small region from Figure 5H.

The localization of PKC α, βII, and ζ isozymes at one and seven days post-injury is shown in Figure 6. At one day post-injury, PKC α was present in the plasma membrane of the PTEC in rat kidneys that underwent one day of reperfusion (Fig. 6A, arrowhead). PKC α was present in the nucleus of the cells that were sloughed into the lumen (Fig. 6A, arrow) of the damaged proximal tubular epithelial cells. A higher magnification of a small region from Figure 6A shows the localization of PKC α in the nucleus (Fig. 6B, arrow). At seven days post-injury, PKC α was found along the plasma membrane of the proximal tubular epithelial cells lining the regenerating tubules and also in identical locations in the cells of the papillary proliferations (p; Fig. 6C). To demonstrate that PKC α was expressed in regenerating proximal tubular cells, serial sections derived from seven-day post-ischemic rat kidneys were stained with either PKC α

Fig. 4. Time course of translocation of PKC α (*A*), βII (*B*), and ζ (*C*) post-ischemic injury to the kidney. Data for each time point are derived from three animals that underwent 60 minutes of ischemic injury followed by different reperfusion times. The intensity of the bands was quantitated as described in the **Methods** section. The intensities of the band derived from sham-operated animals are used as control values, and the data are expressed as percentage of control value for each time point. Data are means ± SE. Symbols are: (△) soluble fraction; (□) particulate fraction.

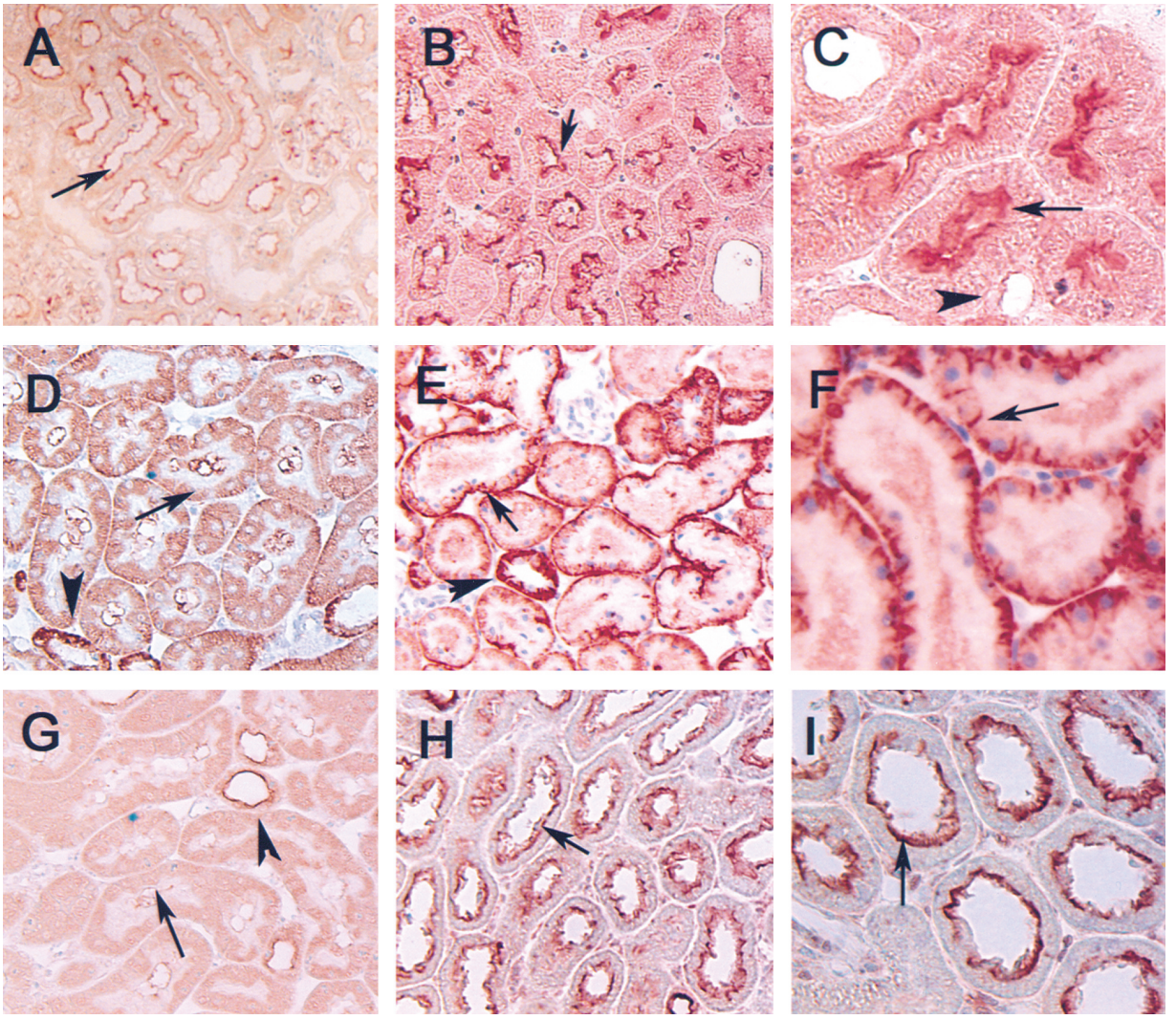


Fig. 5. PKC expression in sham-operated and ischemia-induced rat kidneys. (*A*, *D*, and *G*) Sections derived from sham-operated rat kidneys. (*B*, *E*, and *H*) Kidney sections from rats that underwent 60 minutes of ischemia followed by 10 minutes of reperfusion shown at a magnification of ×250. (*C*, *F*, and *I*) The same kidney sections shown at magnification of ×500. All sections are derived from boundary between the cortex and outer medullary segment. In tissue sections stained with PKC α monoclonal antibody, localization of the isozyme is restricted to the apical and brush border surfaces of the proximal tubule segments (A, B, and C, arrow). PKC βII localizes to the distal tubules in sham-operated rat kidneys (D, arrowhead) and is absent in proximal tubule cells (D, arrow). Its expression, however, is found both in distal (E, arrowhead) and proximal tubules (D and F, arrow) in ischemia-induced rat kidneys. PKC ζ expression is found only in distal tubules in sham-operated rat kidneys (G, arrowhead), and no expression is observed in proximal tubules (G, arrow). PKC ζ expression, however, is induced in the apical segment of the proximal tubules in ischemia induced rat kidneys (H and I, arrow).

(Fig. 6J) or PCNA (Fig. 6K). The staining pattern demonstrates that several of the cells that are positive for PKC α immunoreactivity are also positive for PCNA staining.

In proximal tubules that underwent ischemic damage, the PKC βII isozyme was distributed in the cytoplasm of the PTECs sloughed into the lumen (Fig. 6D, arrowhead) and its distribution is shown at a higher magnification in Figure 6E (arrowhead). In proximal tubules that appear to be not damaged, the distribution was more on the basal and lateral side of the epithelial cell (Fig. 6D, arrow) and shown at a higher magnification in Figure 6E (arrow). However, at seven days post-injury, the expression of PKC βII is found only in certain interstitial cells (Fig. 6F) and was not found in the regenerating tubules.

At one-day following ischemic injury, the ζ isozyme was distributed along the apical side of the damaged PTECs (Fig. 6G, arrow). A higher magnification of one of the tubule is shown in Figure 6H. The apical distribution of the PKC ζ isozyme is shown (Fig. 6H, arrow), and the basal side of the PTEC is shown by the arrowhead. At seven days post-injury, the distribution of PKC ζ showed a similar pattern as that of PKC α (Fig. 6I). It is located

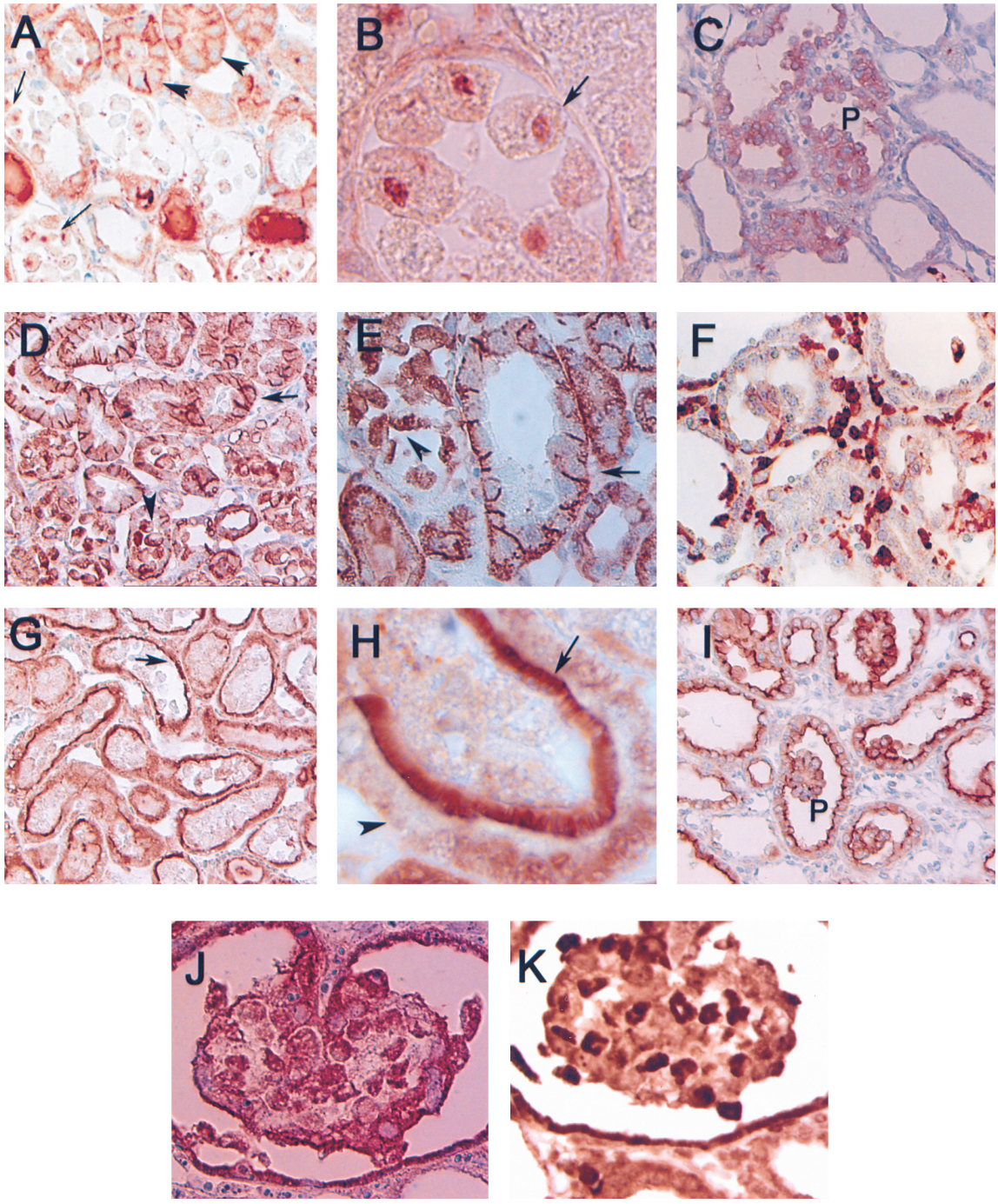


Fig. 6. PKC expression at one day (*A*, *B*, *D*, *E*, *G*, and *H*) and seven days (*C*, *F*, and *I*) after ischemia/reperfusion (I/R) injury. All sections represent outer medullary segments. P, papillary proliferations. PKC α is localized to the plasma membrane of the undamaged proximal tubules (A, arrowhead), but it is translocated to the nucleus in the cells that are sloughed into the lumen of the damaged proximal tubules (A and B, arrow). At seven days post-injury, the expression is found in regenerating cells of the proximal tubule (C) and in papillary proliferations (P). PKC βII is expressed mostly on the basal and lateral side of the plasma membrane of the undamaged proximal tubule (D and E, arrow), while it has a cytoplasmic distribution in damaged PTECs (D and E, arrowhead). PKC βII expression at seven days is localized to certain interstitial cells (F) and not in any regenerating PTECs. PKC ζ expression at one day is along the apical surface of the damaged PTEC (G and H, arrow) The basal side of the PTEC is shown (H, arrowhead). At seven days, the expression is found in the regenerating proximal tubules (I) and in papillary proliferations (P). J and K represent serial sections derived from a seven-day post-ischemic rat kidney. The expression of PKC α (J) and PCNA (K) in the same regenerating papillary proliferation is shown. The degree of magnification in A, C, D, F, G, and I is ×250. Degree of magnification in E, J, and K is ×500. Degree of magnification in B and H is ×1000.

along the plasma membrane of the PTECs lining the regenerating tubules and also in identical locations in the cells of the papillary proliferations (p).

DISCUSSION

This study demonstrates that PKC α, βII, and ζ induced post-ischemic injury to the kidney and further were enhanced during the first hour of the reperfusion period. Their expression was down-regulated after one hour of injury and remained at low levels during 6- to 12-hour time period. The expression level further peaked at one day post-injury and was down-regulated again at two and three days post-injury. An increase in the level of expression was observed further during five to seven days. The increased expression also was accompanied by the translocation of each isozyme from the cytosolic fraction to the particulate fraction at different time points. The translocation of PKC from cytosolic fraction to the membrane fraction is now widely accepted as an index of the enzyme activation [19]. At seven days post-injury, there was almost a complete translocation of PKC α and βII to the particulate fraction, while PKC ζ was found in both fractions. Immunoblotting detected the presence of PKC δ and ϵ in the kidney, but their expression level was not altered by the injury.

Down-regulation of PKC isozymes is a regulatory component of the signal transduction pathways involving PKC. Limited proteolysis of PKC leading to its down-regulation has been described in a model of fetal brain ischemia [20]. PKC α is down-regulated in a dog model of cerebral vasospasm by its limited proteolysis into the 45 kD catalytic fragment [21]. The immunoblotting studies presented here show that a decrease in the 80 kD PKC α at 6 and 12 hours and at 2- to 5-days postinjury is followed by a concomitant increase in its 45 kD proteolytic product.

The intracellular distributions of PKC isozymes were characterized by immunohistochemistry. The expression of PKC α was localized to the apical/brush border membrane of the S2 and S3 segments in the sham-operated rat kidneys as previously reported [22]. Ischemic injury induced the expression of the isozyme in the same segments at early time points (0 to 1 h) during the reperfusion. At one day post-injury, expression of the isozyme was detected at the basolateral surface of the undamaged proximal tubule cell (PTC) in the S3 segment. In proximal tubular cells that are sloughed into the lumen as a consequence of the injury, PKC α was translocated to the nucleus. At seven days post-injury, the isozyme was localized to the regenerating proximal tubular cells, especially those in the papillary proliferations and along the basolateral membrane of the cells that are in the process of migrating and filling up the denuded areas of the basement membrane.

In sham-operated rat kidneys, the expression of PKC βII was localized to the distal tubules. Its expression, however, was enhanced in proximal tubules immediately post-injury and during reperfusion. The PKC βII isozymes translocated to the plasma membrane of the cells that are sloughed off into the lumen at one day post-injury. At seven days post-injury, the expression of the PKC βII isozyme was localized to certain interstitial cells. Expression of the PKC βII isozyme was described in the cortical and medullary interstitial cells of adult rat kidneys [22]. A role for both PKC βII and interstitial cells in growth and regeneration has previously been described [23, 24]. The induced expression of PKC βII in the interstitial cells adjacent to the regenerating tubules suggests a possible role for the isozyme in the regeneration process of the damaged proximal tubules.

In an earlier report, La Porta and Comolli showed by immunoblot analysis that an increase in PKC α occurred at 60-minutes postinjury, but no increase in the immunoreactivity of PKC β and ζ was observed [11]. At two hours of reperfusion, an increase in PKC β activity was observed. The expression of PKC δ was down-regulated during the first 15 minutes, but was up-regulated at 30 and 60 minutes. PKC ϵ expression was reported to be increased at early time points and at 60 minutes post-injury [11]. Our studies demonstrate that there is early induction of PKC α, βII, and ζ in rat kidneys during the first hour after ischemic injury. Increased expression of PKC α, βII, and ζ was also observed at one and five to seven days post-injury. The variation in the expression pattern of the various isozymes between the present study and the previous report may be partly due to the difference in the severity of the injury (30 vs. 60 min) utilized in the studies. It should also be noted that immunoblot analysis for PKC βII was not performed in that study.

Currently, there is little knowledge relating to the role of PKC or the identities of physiological substrates of PKC in renal ischemia. A role for PKC isozymes in ischemia/reperfusion injury has been implied in the heart and brain based on the alteration of its activity post-injury. PKC α is reported to be induced and autophosphorylated following hypoxic and radiation stress [25]. The enzyme is found to be translocated mainly to the nuclear fraction following both types of stress. The identities of the target proteins that are phosphorylated following translocation of PKC α to the nucleus are not known.

A better understanding of the regulation, activation, and key substrates of individual PKC isozymes in renal ischemia may provide ways to intervene pharmacologically in PKC-mediated molecular pathways that could lead to effective treatment for acute renal failure.

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